

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

1. (Currently Amended) A method of enumerating the number of CD4+ lymphocytes in a cell sample, comprising:

~~a) determining the absolute number of CD45+ cells in a cell sample using a hematology analyzer having a facility for fluorescence measurement or a flow cytometer;~~

~~b) a) determining the proportion of CD45+ white blood cells which are CD4+ lymphocytes using fluorescence measurement via flow cytometry by:~~

- ~~(i) establishing a primary discriminator to discriminate defining a primary population of all CD45+ white blood cells of interest;~~
- ~~(ii) establishing defining a secondary discriminator population, within said primary population, of all discriminator to discriminate CD4+ lymphocytes having CD4^{Bright}/low side scatter expression; and~~
- ~~(iii) calculating the proportion of CD45+ white blood cells which are CD4+ lymphocytes; and~~

~~b) determining the absolute number of CD4+ lymphocytes in the cell sample by:~~

- ~~(i) directly counting the proportion calculated in step a)(iii); or~~
- ~~(ii) counting the number of CD45+ white blood cells in the primary population defined in step a)(i) and multiplying this number by the proportion calculated in step a)(iii) multiplying the absolute number of CD45+ cells obtained in step (a), by the proportion of CD45+ cells which are CD4+ lymphocytes, obtained in step (b), thereby determining the absolute number of CD4+ lymphocytes in the cell sample.~~

2. (Currently Amended) The method according to claim 1, wherein step ~~a)(i)(a)~~ determining the absolute number of CD4+ cells in the cell sample is performed by flow cytometry using a bead-based or volumetric-based counting method.

3. (Withdrawn) A method according to claim 1, wherein a white blood cell differential is further identified from the total CD45 expressing population identified in step (a) of claim 1, and further comprising the steps of:

- d) (i) determining the percentage of CD4 monocytes as a function of the total CD45 expressing population identified in step (a); and
(ii) calculating the number of CD4 monocytes in the sample by multiplying the percentage of CD4 monocytes determined in step (e(i)) by the number of CD45 cells obtained in step (c);
- e) (i) determining the percentage of CD4 eosinophils as a function of the total CD45 expressing population identified in step (a); and
(ii) calculating the number of CD4 eosinophils in the sample by multiplying the percentage of CD4 eosinophils determined in step (f(i)) by the number of CD45 cells obtained in step (c);
- f) (i) determining the percentage of CD4 negative granulocytes as a function of the total CD45 expressing population identified in step (a); and
(ii) calculating the number of CD4 negative granulocytes in the sample by multiplying the percentage of CD4 negative granulocytes determined in step (g(i)) by the number of CD45 cells obtained in step (c);
- g) (i) determining the percentage of CD4 negative lymphocytes as a function of the total CD45 expressing population identified in step (a); and
(ii) calculating the number of CD4 negative lymphocytes in the sample by multiplying the percentage of CD4 negative lymphocytes determined in step (h(i)) by the number of CD45 cells obtained in step (c);
- h) (i) adding the percentage of CD4 lymphocytes identified in step (b) and the percentage of CD4 negative lymphocytes obtained in step (h(i)) to obtain the percentage of total lymphocytes;

- (ii) determining the percentage of total lymphocytes as a function of the total CD45 expressing population identified in step (a); and
- (iii) calculating the number of total lymphocytes in the sample by multiplying the percentage of total lymphocytes obtained in step (i(ii)) by the number of CD45 cells obtained in step (a).

4. (Withdrawn) A method according to claim 1, further comprising the steps of:

- d) determining the percentage of basophils as a function of the total CD45 expressing population identified in step (a); and
- e) calculating the number of basophils in the sample by multiplying the percentage of basophils determined in step (j) by the number of CD45 cells obtained in step (c).

5. (Currently Amended) A method according to claim 1, wherein the cell sample is whole unlysed blood, unfractionated, fractionated or lysed whole blood.

6. (Withdrawn) A kit including CD4 and CD45 antibodies for use in enumerating the number of CD4 cells in a sample.

7. (Withdrawn) A kit according to claim 6, which further includes instructions for performing the method of enumerating the number of CD 4 cells in a cell sample.

8. (Withdrawn) A kit according to claim 6, which further includes one or more reagents selected from the group consisting of a red cell lysating agent, a stabilizer, a fixative, control cells, media and bead reagents.

9. (Withdrawn) A machine readable medium comprising instructions, which when executed by a machine, cause the machine to perform the method steps of claim 1.

10. (Withdrawn) A machine readable medium according to claim 9, which is configured for use in conjunction with a flow cytometer and/or haematology analyser.

11. (Withdrawn) A machine readable medium according to claim 9, which includes instructions for performing analysis methods selected from the group consisting of impedance, light scatter, fluorescence and precision volume counting.

12. (Withdrawn) The method of claim 1, wherein the method includes the step of enumerating the number of CD4+ lymphocytes or CD4+ T-cells in a cell sample from the patient with HIV or other immune deficiency condition or disease; and wherein the method is used to monitor the immune status of the patient.

13. (Withdrawn) A method according to claim 12, wherein the patient's immune status is determined or monitored to determine the patient's response to antiretroviral treatment.

14. (Currently Amended) The method according to claim 2, wherein the bead-based counting method comprises adding a known numbers of beads to the cell sample; and counting the beads and cells simultaneously to obtain the absolute number of CD45+ cells count.

15. (Currently Amended) The method according to claim 1, wherein ~~the number of CD4+ lymphocytes is enumerated in a dual platform approach whereby the~~ primary population of all ~~number of CD45+ white blood cells determined defined in step (a)-a)(i) is~~ obtained using a hematology analyzer, and the ~~proportion of secondary population of all CD4+ lymphocytes having CD4^{Bright}/low side scatter expression defined~~ determined in step (b)-a)(ii) is performed-obtained using a flow cytometer.

16. (Currently Amended) The method according to claim 1, wherein the primary population of all CD45+ white blood cells is defined in step a)(i) ~~number of CD45+ cells is determined in step (a)~~ per volume of blood.